INFORMATION 1 Details about specimen photography

Kaoru Kuriiwa

■Choice of camera and lens

1. Compact or SLR camera

The most commonly used cameras can be divided into 2 main types: (1) compact digital camera, which has an integrated lens and (2) SLR camera, which has a replaceable lens. The question is which of the 2 types is suitable for specimen photography.

The basis of selecting a compact camera will obviously be convenience. A compact camera is small, light, and can be used anywhere. No complicated setup is required, and most people can easily handle it. Basically, it captures a deep-focus shot (everything from the objects in the background to those in the foreground are evenly focused), without adjusting the aperture or shutter speed. On the other hand, the lens of an SLR camera needs to be replaced depending on the purpose; furthermore, the body is large and heavy as compared with that of a compact camera. Exposure needs to be set according to the aperture and shutter speed, and the depth of field (the depth and thickness that come into focus) should be carefully selected. Thus, it requires some knowledge and skill to take a good photograph. The performance of both types of cameras is improving with time, but there still exist significant differences in 2 basic parts, irrespective of the extent of sophistication of the machines. The difference is in the size of the light-receiving element (sensor) and that of the aperture of the lens.

A camera receives optical information through the sensor, and there is considerable difference in the sensor size between compact and SLR cameras. Suppose there are 2 cameras—a compact camera and an SLR camera—with the same resolution, *i.e.*, ten megapixels (10 MP). In a 10-MP camera, the optical information received by the sensor is delimited into 1 × 106 dots, and an image is produced. The sensor of a commonly used compact camera is 5.7 mm × 4.3 mm (1/2.5-inch-type charge-coupled device [CCD] sensor), whereas that of a general SLR camera is approximately 24 mm × 16 mm (advanced photo system [APS] sensor). As evident, there is a difference of more than 15 times. In fact, despite both being 10-MP cameras, there is a significant difference in the optical information of each pixel. Thus, an SLR camera has more optical information than a compact camera, allowing it to duplicate the object faithfully and describe it in more detail. Imagine sharing a table-sized cake and a 20-cm-wide cake, each with 10 people. It is needless to say a piece of which cake will be larger (consider cake size as sensor size, number of people as pixel value, and quantity of the cake as optical information). Furthermore, there is a difference in the bore diameter of the lens between compact and SLR cameras, and an SLR camera, which has a larger bore diameter, has higher resolution.

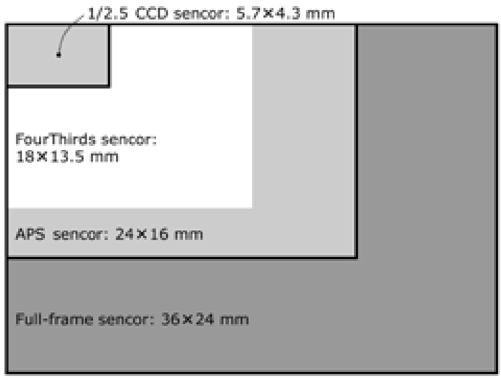

Figure 1. Comparison of the light-receiving element (sensor) of compact and SLR cameras.

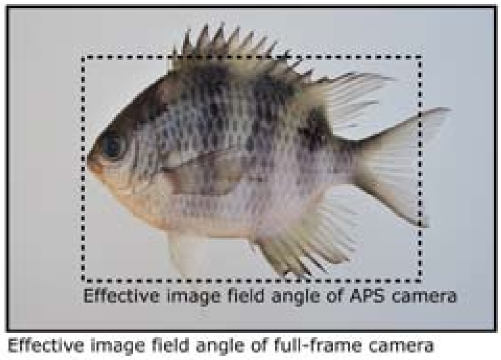

Figure 2. Difference in the effective image field angles of full-frame and APS cameras.

Specimen photography for academic purpose should be taken under better photographic equipments to take a high-resolution image. Therefore, it is preferable to use an SLR camera.

2. APS or full-frame camera

There are actually 3 main sensor sizes for digital SLR cameras (Figure 1). One is full-frame sensor, the size of which is the same as that of the sensor of a 35-mm film camera (full-frame camera). Another is APS sensor, the size of which is the same as that of the sensor of an APS camera. The third type is called FourThirds sensor, which is mainly adopted by OLYMPUS. Full-frame sensors have so far been adopted only by a few companies (*e.g.*, Nikon, Canon, and SONY) in their top models, and APS sensors are adopted by other companies in their SLR cameras. Here, we focus on full-frame and APS cameras.

Full-frame and APS cameras have different field of view (field angle) even when lenses with the same focal length are used (Figure 2). This is due to the difference in sensor size. For example, when a lens with 50-mm focal length is used, the field angle of a full-frame camera becomes 50 mm, but that of an APS camera becomes approximately 75 mm, which is 1.5 times smaller (effective image field angle). In general, field angle is expressed using that of a 35-mm film camera (full-frame camera) as a reference. That is why the field angle of an APS camera lens is often indicated as "35-mm camera conversion: __ mm." In other words, if a 50-mm focal length lens is used in an APS camera, the field angle corresponds to that of a 35-mm film camera with an approximately 75-mm focal length lens. However, the field angle is not actually reduced by 1.5 times, but the visible 50-mm field angle of a full-frame camera is reduced to make it approximately 75 mm for an APS camera.

Whether the photographer chooses a full-frame or an APS camera depends on his preference based on the characteristics of both. The advantages of a full-frame camera include high sensitivity because of its large sensor, broad range of tones, and wide field angle. However, it is expensive, large in size, and may produce limb darkening. On the other hand, the advantages of an APS camera include telephotography, ability to achieve high resolution in the center of an image because of reduction of field angle, small size, light weight, and cost effectiveness. Its disadvantage is that the focal length of the lens is not the same as the field angle, but is a 35-mm format equivalent (for example, a 50-mm focal length lens cannot be used for 50-mm field angle). Further, to get the same aperture value from the same effective image field angle, the aperture diaphragm in a full-frame camera needs to be adjusted to narrow down the aperture by about one level. In short, it should be noted that there is a difference in the depth of field at the same aperture level (f-number, described later with Figure 8).

3. Zoom or micro lens

There are 2 kinds of lenses: zoom lens and fixed-focal-length lens. The difference lies in that the focal length of the former can be changed, whereas that of the latter is fixed. Zoom lens is very convenient because it does not require the position of the camera to be changed to change its focal length. However, it should be noted that its release f-number (maximum aperture, described later with Figure 8) is generally darker and its resolution is lower than that of fixed-focal-length lens. Dark release f-number is disadvantageous for specimen photography and often requires narrowing down of the aperture. Since resolution is important in specimen photography, low resolution of zoom lens is also a disadvantage. In addition, zoom lens can best reduce aberration around the center of the focal area; the aberration increases at the edges of the focal area (barrel distortion occurs on the wide-angle side, and pincushion distortion occurs on the telescopic side). Thus, fixed-focal-length lens, called micro lens, appears to be the best choice for specimen photography.

The characteristics of micro lens are as follows: (1) high resolution in close-up photography, (2) high magnification (can photograph with life-size or increased magnification), and (3) minimum aberrations (especially distortion). In terms of resolution of a close-up photograph, micro lens shows much better performance than zoom lens. There are micro lenses with zoom function (zoom micro lenses) and zoom lenses with micro function, but they are quite inferior to fixed-focal-length micro lenses. In addition, the maximum magnification of micro lens is 1:1 (life-size magnification) or 1:2, while that of zoom lens with micro function is only approximately 1:4.

Many companies sell various kinds of micro lenses such as standard (focal length, approximately 50 mm), medium telephoto (focal length, 100 mm), and telephoto (focal length, 150 mm or more). In specimen photography, the suitable focal length of lens differs depending on the size of the specimens. This is because overhead photography is conducted using a tripod and camera base. Furthermore, shooting distance is an important factor. The distance between the sensor and the subject is called shooting distance (Figure 3). A lens with shorter focal length has a longer minimum shooting distance, and vice versa. When a large specimen is photographed using a lens with long focal length, the field angle lacks width (short

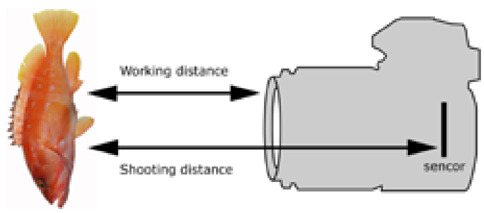

Figure 3. Shooting distance and working distance.

Figure 4. Difference in the effective image field angles of full-frame and APS cameras. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/16).

Figure 5. Difference in the effective image field angles of full-frame and APS cameras. Nikon D60, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/8).

shooting distance), and the image does not fit in frame. This should be borne in mind that the actual field angle (effective image field angle) of an APS camera is converted into 35-mm format. The distance from the front of the lens to the object is called working distance (Figure 3). The description of lens performance by a company mentions the minimum shooting distance, but not working distance. Note that even though the minimum shooting distance is the same, working distance can be different among lenses with different body length. In addition, to what extent a lens can enlarge the image of an object is expressed as the maximum magnification, and this is not related to focal length. When lenses with the same maximum magnification and different focal lengths are compared, the difference is found to be only in the shooting distance for maximum magnification.

As mentioned above, the effective image field angle of an APS camera becomes equal to that of 90-mm medium telephoto macro lens in 35-mm format, even though standard macro lens is used. When a specimen measuring more than 40–50 cm is photographed using 90-mm medium telephoto macro lens, the image does not fit in frame because of short shooting distance (Figure 2). Figures 4 and 5 show how difference in field angle due to difference in sensor size affects the actual photograph. The photographs are taken with a full-frame camera and an APS camera, with both fixed in position and having the same focal length lens (60 mm) (standard length of the specimen: 26 cm). Figure 4 shows the photograph taken with a full-frame camera, and the effective image field angle is 90 mm in 35-mm format. To photograph the same area as in Figure 4 by using an APS camera, a lens with 40-mm shooting distance (35-mm format equivalent: 60 mm) needs to be used or the camera needs to be positioned comparatively high. If a large specimen is taken, an APS camera will not photograph it as a full-frame camera because of narrow field angle. If an even larger specimen is taken, macro lens will not allow its image to fit in frame, regardless of which camera is used. In this case, zoom lens should be used. As stated above, aberrations of zoom lens increase at the edges of the focal area. Therefore, with zoom lens, the photograph should be taken being as close to the center of the focal area as possible.

■Shooting procedure and settings

1. Storage formats of digital images

Photographs taken with a digital camera are stored in RAW, TIFF, and JPG formats. RAW format, as the name suggests, is a raw image data file. It is “developed” into image files in TIFF and JPG formats. The word “developed” implies that a RAW file is “converted” into a TIFF or JPG file on the computer. A TIFF image does not deflate during development; thus, the image quality is good, although the file size increases. On the other hand, a JPG image is compressed during development; thus, the file size decreases but the image quality is low. As explained, each format has its own characteristics. The question is which is the best format for storing specimen photographs.

As stated above, a RAW file contains raw image data, and the image is simply a digitized output signal from the sensor. The data are not adjusted by the image processor engine of the camera, and hence, the image can be retouched without deteriorating its quality. On the other hand, TIFF and JPG files contain processed image data, and hence, the image quality deteriorates with every retouch. Allegorically, raw ingredients collected for cooking correspond to a RAW image, and cooked food corresponds to TIFF and JPG images. The concept is the same that cooked food cannot really be re-cooked.

Many characteristics of a RAW image, such as exposure level, white balance, noise, and color tone, can be changed; thus, RAW format is undoubtedly more flexible than TIFF and JPG formats. If retouch is presupposed, the image should definitely be saved in RAW format. If a specimen photograph is to be used in a research paper, the background often needs to be processed and the photograph size also needs to be changed. For this purpose, the image should be saved in RAW format. On the other hand, data in RAW format are very voluminous and require extra effort for conversion into JPG format. Therefore, when many specimen photographs are taken, the images may be saved in JPG format, and only when it is decided beforehand that a particular image has to be used in research papers, it may be saved in RAW format. In terms of tint of specimen photographs, white balance is very important. This point needs to be considered while saving an image in JPG format.

2. Camera installation and lighting

There are mainly 2 ways of placing a specimen for photography: (1) the specimen can be placed in a transparent glass tank filled with water (Figure 6) and (2) the specimen can be placed on anti-reflex glass and photographed on land (Figure 7). Fishes with many skin flaps, such as those of Scorpaenoidei and Lophiidae, should be photographed in water in order to correctly visualize the dimensions of the skin flaps. A very large specimen that cannot fit in the aquarium should be photographed by the latter method.

When an immersed specimen is photographed, lighting plays a very important role. Shine flashlights from either side of the specimen, taking care that there is no reflection or shadow on the fish body. It often works well if a tracing paper is fixed in front of the light to soften it. Measure the exposure with the flashlights on, and photograph the specimen with only the flashlights on and the room lamp off. Suitable background color—whether black or white—should be decided depending on the skin and scale color of the fish, although the best way is to photograph the specimen with both backgrounds. Furthermore, if there

Figure 6. Photography technique for a specimen placed in water.

Figure 7. Photography technique for a specimen placed on land.

is air inside the abdominal cavity, the specimen may float on the water surface or heel over in the aquarium. In this case, puncture the right side of the abdomen with a surgical knife to remove the air. Be careful not to damage the organs, so that the water remains clean. If an organ is accidentally damaged, wash with running water. A glass aquarium is better than an acrylic aquarium, because it has resistance against scratches and tarnish.

For photography on land, the specimen should be placed outdoors in a shaded area. Even in a shaded area, sometimes a different shade of the specimen may be recorded. This should be taken care of. Pay attention to white balance too, and configure the settings such that, as much as possible, the real color of the specimen is recorded. Mist the fish frequently with water to prevent dehydration. However, wipe the body surface lightly while photographing in order to avoid refection due to the water adhering to the body surface.

3. Minimization of shaking

Shaking should be minimized while photographing. For this purpose, a tripod or camera base and remote release should be used. If possible, use a camera level vial to position the camera horizontally. It has earlier been stated that the lens aperture often needs to be narrowed down, and this decreases shutter speed (described later with Figure 8). Slow shutter speed increases camera shake, and therefore, photographs should not be taken with a handheld camera. Nowadays, many companies equip cameras with a function of hand-shake correction. However, this function can only “alleviate” hand shake, and there is a limit of the degree of correction. In specimen photography, slow shutter speed exceeds the limit of the hand-shake correction function. Further, hand-shake correction should be turned off while using a tripod, because it will cause another shake if kept turned on. Release is used to avoid shake when the shutter button is pushed. If release is not available, a self-timer (set at approximately 2 s) or a remote control can be used.

An SLR camera is equipped with an in-built mirror. At the moment when the shutter button is pushed, the mirror flips up and light is received by the sensor behind the mirror. The flipping up of the mirror generates a slight shake (mirror shock). This shake cannot be avoided by using a tripod, camera base, release, self-timer, or even the hand-shake correction function (this is a different kind of shake). To avoid mirror shock, raise the mirror before pushing the shutter button. This is called mirror lock-up photography and is a very good technique for specimen photography. However, all cameras do not have the feature of mirror lock-up photography. Incidentally, Nikon provides the exposure-delay mode (not available in lower models). With this mode activated, the mirror flips up when the shutter button is pushed, and then after approximately 0.4 s, the shutter is released. This, like mirror lock-up photography, is a method to control mirror shock.

■Photography

1. Exposure

Exposure depends on aperture and shutter speed. The aperture value, which correlates with the depth of field, is very important. Therefore, exposure is decided using manual setting or aperture priority autosetting. In manual setting, the photographer decides the exposure by setting the aperture value and shutter speed. In aperture priority autosetting, the photographer decides the aperture value, and then, the camera automatically decides shutter speed that suits the aperture. Please decide the correct exposure, referring to the histogram.

Figure 8 shows a correlation chart of aperture, depth of field, light entering the aperture, and shutter speed. The aperture value (f-number) starts from 1 and increases in multiples of 1 and √2 (1.4) in sequence (that is, a sequence of multiples of 1 and 1.4 in alternation). As the aperture is narrowed down by one level, the amount of light entering it decreases 2 times. Accordingly, for the same exposure after narrowing down the aperture by one level, shutter speed should be decreased to half (shutter speed should be expressed as multiples of 2).

Overexposure (too much light) causes clipping, and underexposure (too less light) . Be careful that clipping particularly tends to occur with a digital camera. If an image is saved in RAW format, the clipped or suppressed part can be retouched, and information can later be retrieved (this technique cannot be adopted with an image saved in JPG format). However, if an image is completely white or black, the color in each part is saturated, and in this case, no information can be retrieved even from RAW format. The view through the viewfinder differs with each camera model and manufacturer. Therefore, a few photographs should be taken at each exposure level from light to dark. It is also useful to use the Auto Bracket function, with which a camera automatically takes a few photographs at each exposure level (this function is called the automatic exposure bracketing [AEB] function in Canon).

2. Aperture and depth of field

Fishes have body width. Hence, they should be photographed by narrowing down the aperture to increase the depth of field (Figure 8). In a general portrait, the focus is on the eyes; similarly, in a fish specimen photograph, the focus should be on the eyes of the fish. If the depth of field is not enough and each fin is not in focus, the specimen photograph cannot be used for academic purpose. Therefore, the aperture generally needs to be narrowed down. Note that if the aperture is narrowed down too much, resolution becomes low because of diffraction of light.

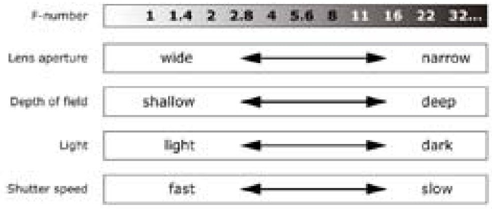

Figure 8. Correlation chart of aperture, depth of field, light entering the aperture, and shutter speed.

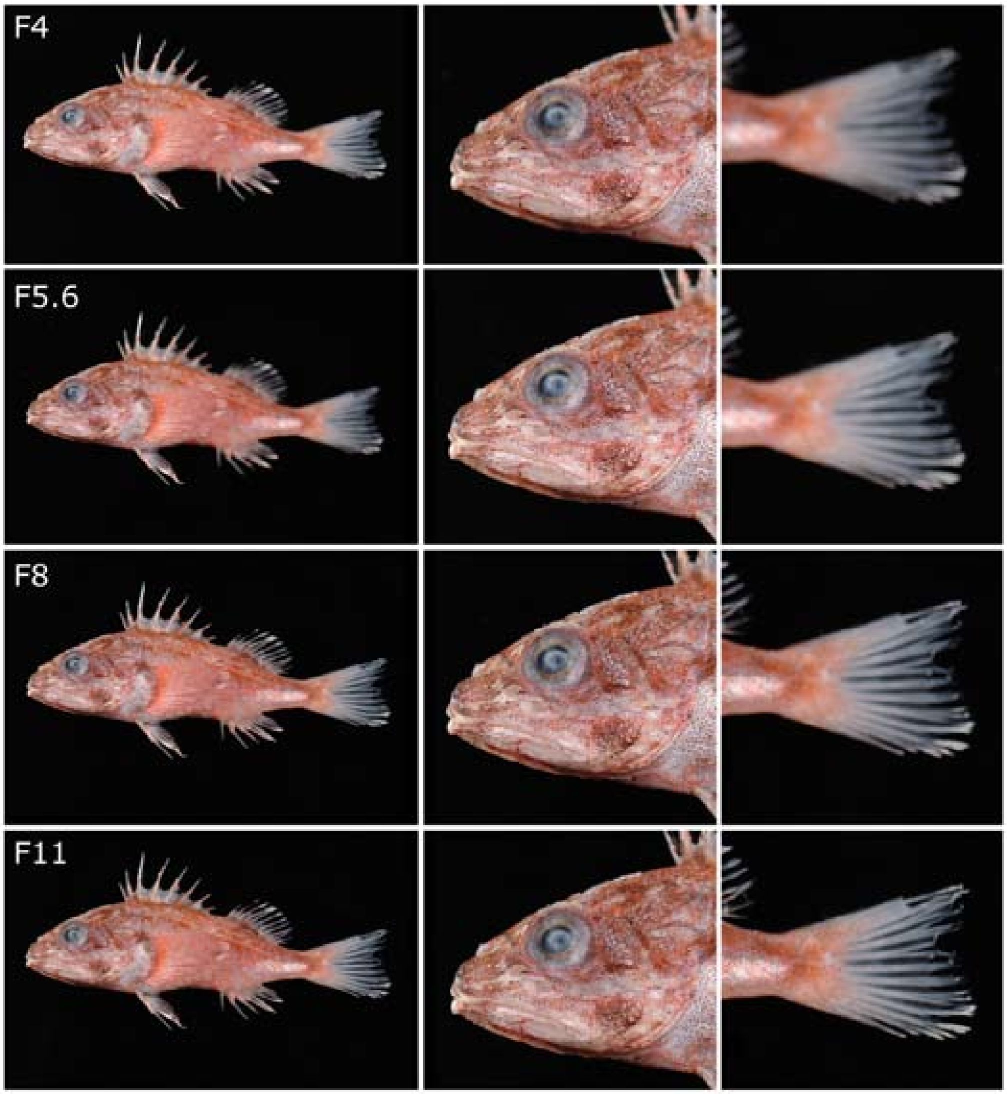

Figure 9. Difference in depth of field depending on f-number. Standard length of the specimen, 5.6 cm; total length of the specimen, 7.1 cm; shooting distance, approximately 30 cm. Nikon D3, AF-S Micro Nikkor 105mm f/2.8G.

This is called narrow-aperture defocus. When the aperture is narrowed down, the path of light entering the lens becomes narrow. If the aperture is narrowed down too much, the light travels around the back of the aperture diaphragm, and because of interference of this light, resolution becomes low. Diffraction occurs independently of the camera, whether film or digital, but the effect is more notable in the case of a digital camera. This is because the smaller the sensor is, the more it can be affected by diffraction. An APS camera is particularly affected severely. Degradation of resolution due to diffraction can be interpreted as degradation of contrast, to be precise. In other words, a photograph in “white - black - white” changes to one in “white - gray - white.”

Depth of field depends not only on aperture but also on shooting distance. Long shooting distance increases the depth of field, whereas short shooting distance decreases it. That is why the depth of field

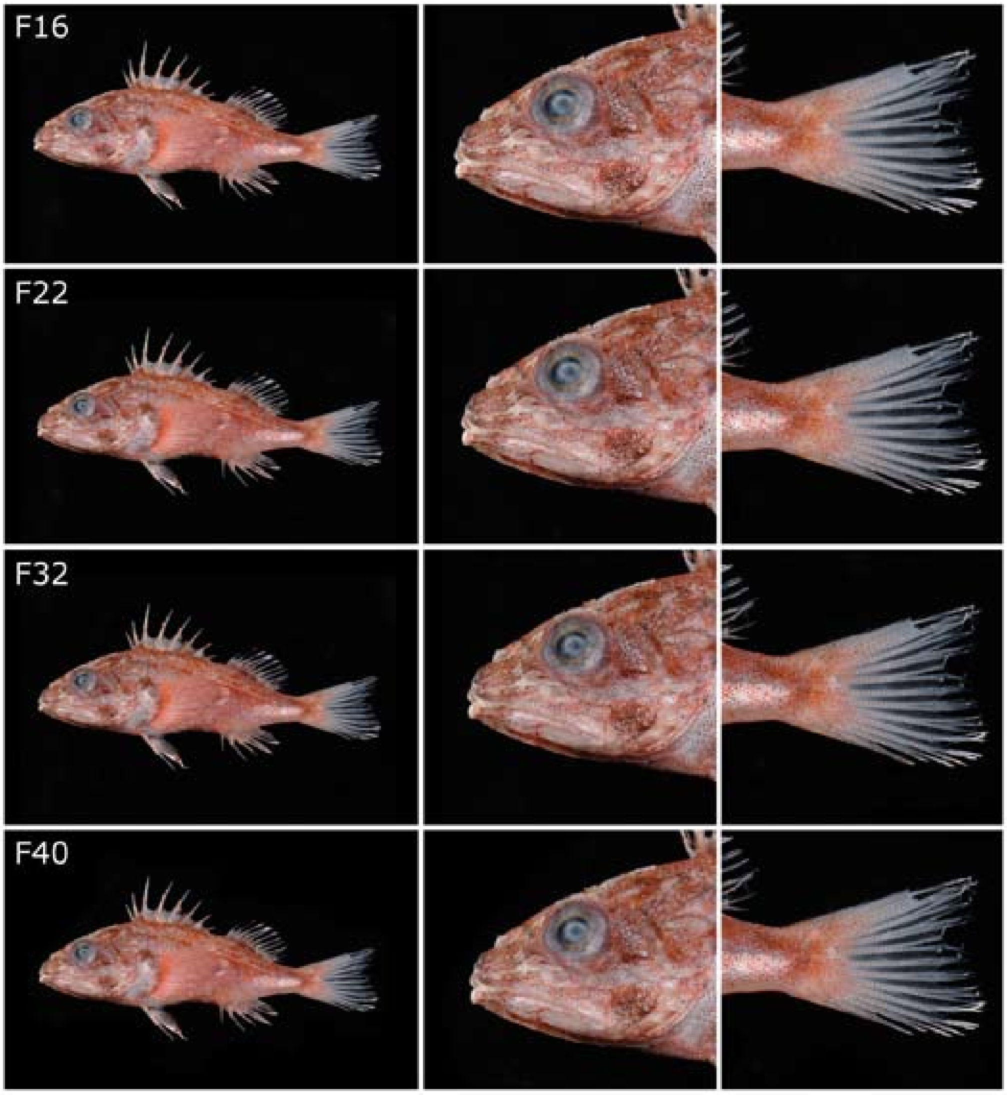

Figure 10. Difference in depth of field depending on f-number. Shooting conditions and equipment are the same as indicated for Figure 9.

becomes extremely shallow in close-up photography, in which macro lens is used. The resolution peak of the lens itself is generally at f/5.6–f/8; however, although the fish eyes or head are in focus around that f-number, the caudal fin and soft rays of the anal fin sometimes appear blurred. In this condition, the aperture may need to be narrowed down to as much as f/32. However, this decreases the lens resolution, and at the same time, degradation of resolution also occurs because of diffraction due to an excessively narrowed aperture. Check the focus by scaling up the viewfinder, and look for the appropriate f-number and depth of field. The depth of field also depends on focal length. The shorter the focal length is, the deeper the field becomes (pantoscope), and the longer the focal length is, the shallower the field becomes (telescope).

3. Examples

Let us take a look at examples to un-

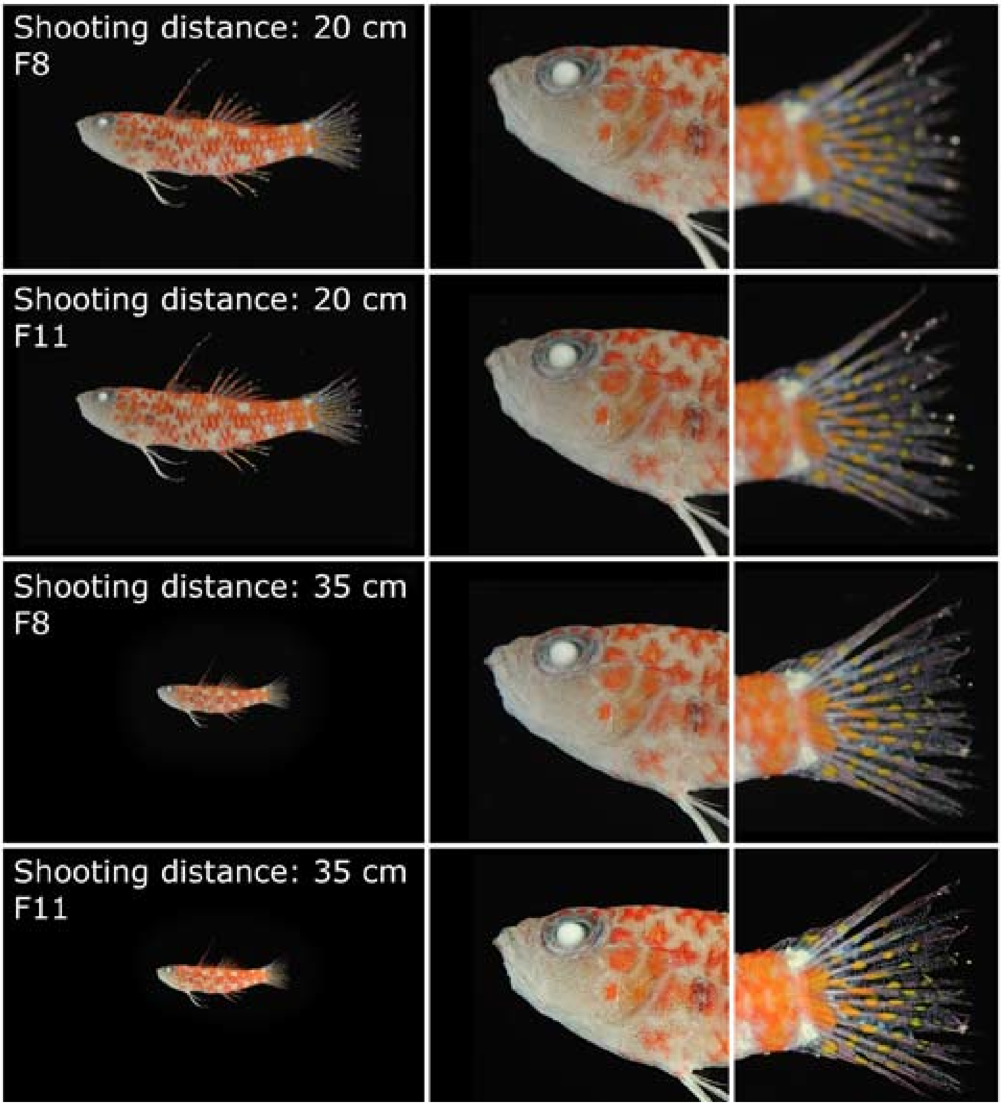

Figure 11. Difference in depth of field depending on f-number and shooting distance. Standard length of the specimen, 2.2 cm; total length of the specimen, 2.7 cm; shooting distance, approximately 20 or 35 cm. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G.

derstand the correct f-number and depth of field for photographing a specimen. Figures 9–11 show photographs taken with a full-frame camera. The f-number of a full-frame camera is about one level lower than that of an APS camera, which has a different sensor size, to generate the same aperture values from the same effective image field angle. Thus, in the following explanation, f/8 of a full-frame camera corresponds to f/5.6 of an APS camera.

Specimen photographs taken at f/4–f/40 (APS: f/2.8–f/36) are shown in Figures 9 and 10. The standard length of the specimen is 5.6 cm (total length, 7.1 cm), and in all the photographs, the focus is on the eyes. This is almost the limit of close-up photography (shooting distance, approximately 30 cm). The left side shows the complete image; the middle shows the image at the same f-number, with the cephalic part cropped; and the right side shows the image with the caudal fin cropped as well. The photograph obviously lacks the depth of field at f/4–f/8 (APS: f/2.8–f/5.6). The camera is beginning to focus on the caudal fin at f/11 (APS: f/8), and focuses it enough at f/22 (APS: f/16). In the cephalic part, on the other hand, degradation of resolution due to diffraction becomes noticeable at f/22 (APS: f/16). It is particularly notable at f/40 (APS: f/36). The effects of the depth of field and diffraction are only noticeable in the original image in large size, not when the image size is scaled down as shown here. Nevertheless, even in the reduced image, the difference in the depth of field is quite obvious. Recent digital camera models may be able to control the effect of diffraction well. Figure 11 shows the photographs taken with the lens at two distances and f-numbers from a small specimen (shooting distances, 20 and 35 cm; f-numbers, f/8 and f/11). When the distance from the object was increased, large depth of field was possible even with small f-number.

Small fishes, *e.g.*, a few centimeter long ones, should be photographed from a very close distance, so that they occupy the full frame. Therefore, the optimal f-number tends to be large. On the other hand, a large fish needs to be photographed from a long distance, and hence, the aperture need not be narrowed down too much to obtain the optimal depth of field. Figure 12 shows the photograph of a specimen with less body depth (standard length, 18.6 cm; total length, 22.7 cm), taken with a full-frame camera (shooting distance, approximately 56 cm). The aperture value is f/11 (APS: f/8). Figure 13 shows the photograph of a specimen with greater body depth (standard length, 13.9 cm; total length, 17.1 cm), taken with an APS camera (shooting distance, approximately 68 cm). The aperture value is f/16 (full-frame: f/22).

The examples mentioned above indicate that the optimal f-number for fishes longer than a few centimeters is mostly acceptable up to f/22 of a full-frame camera and about f/16 of an APS camera (microscope photography is better for fishes smaller than a few centimeters, not SLR photography). Body depth differs depending on species. Therefore, every time a photograph is taken, the view through the viewfinder should be checked to ascertain the optimal f-number. With practice, one can roughly estimate the standard value from the specimen size, body shape (body depth), and shooting distance before pushing the shutter button.

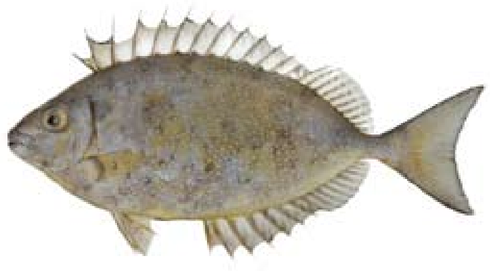

Figure 12. Specimen without much depth. Standard length, 18.6 cm; total length, 22.7 cm; shooting distance, approximately 56 cm. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/11).

Figure 13. Specimen with good body depth. Standard length, 13.9 cm; total length, 17.1 cm; shooting distance, approximately 68 cm. Nikon D60, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/16).